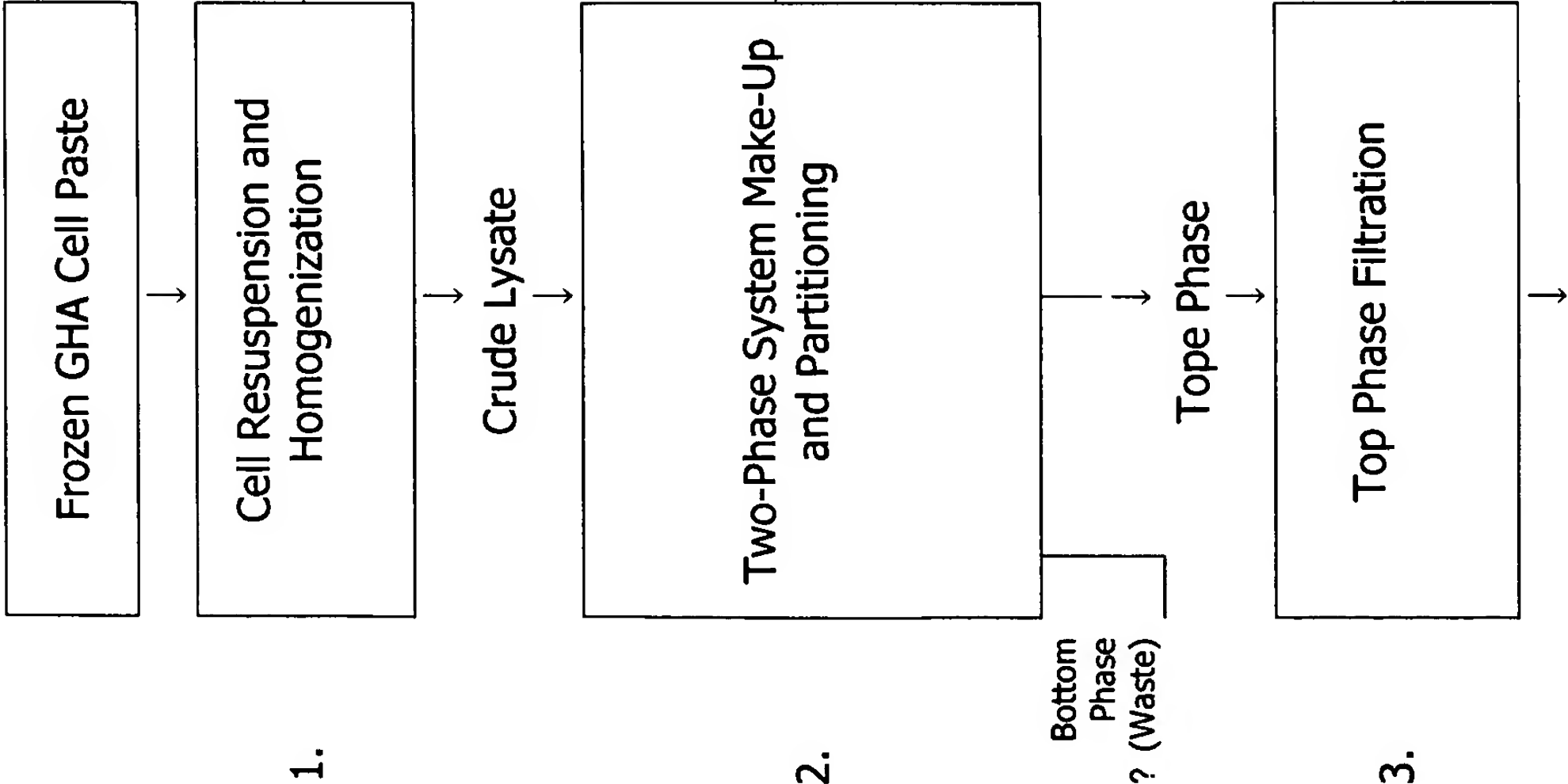


Figure 2 a

UNIT OPERATION

PROCESS DESCRIPTION

PROCESS CONTROLS



Follow procedure of U.S. Pat. No. 5,849,535 (recombinant expression of B-2036, centrifugate and freeze)

Cell Past Mass: 163-183 kg
Buffer: 150 mM Tris and 5 mM EDTA, pH 7.2 (6.14 L/kg cell paste)
Homogenizer Pressure: 900-1000 Bar
Feed Temperature: </=15°C

Final Volume: ~1065 - 1136 L
Monitor OD550 during cell resuspension

Conditioning: 125 grams ammonium sulfate per liter crude lysate
125 grams PEG4600 per liter crude lysate
Phase Contacting Temp: 24-33°C
Phase Contacting Time: 1-2 hours with mixing
Separation Method: Continuous disk stack centrifuge
Speed: 8340 RM
Inlet Feed Rate: 7 LPM

Monitor conductivity of extract.

Dilution of Top Phase 1:1 with USP Purified Water
Serial Filtration
1) Charged delipidating depth (Seitz or equivalent)
2) Charged cellulose depth (Seitz or equivalent)
3) 0.2 µm absolute cellulose acetate

Pre-Filter: LAL Bioburden, RPHPLC
Post-Filter: LAL Bioburden, RPHPLC, IEX-HPLC, HIHPLC
Specific Recovery: >/= 2.5 g B-2036 per kg cell paste

Figure 2 b

UNIT OPERATION

PROCESS DESCRIPTION

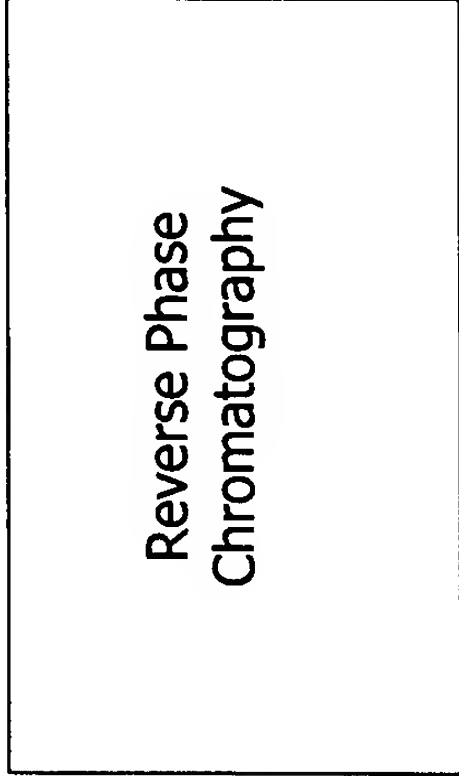
PROCESS CONTROLS

Filtrate



Reverse Phase
Chromatography

4.



Eluate 1



Ion Exchange
Chromatography
(Flowthrough Mode)

5.

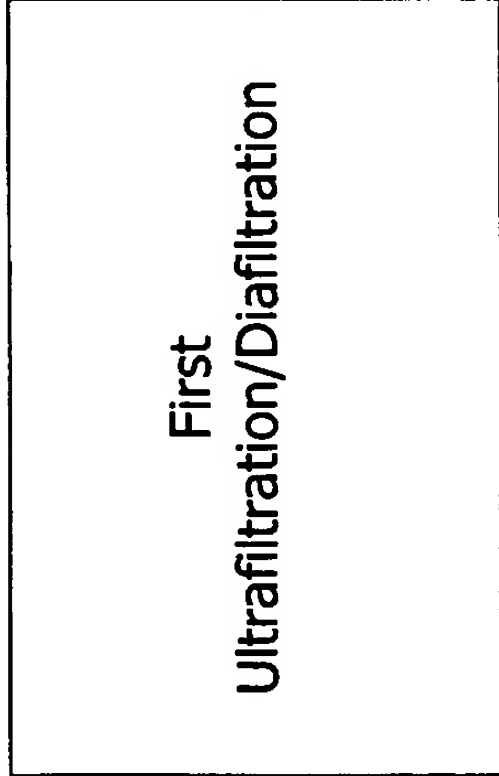


Flowthrough



First
Ultrafiltration/Diafiltration

6.



Permeate
? (Waste) 1

Resin Type: Polymeric Methacrylate Reverse-Phase (Amberchrom CG71M)

Inline 0.2 µ filtration of load
Gradient: Linear, 0-100% B, 10 CV
Buffer A: 50 mM Tris, pH 7.2
Buffer B: 50% Hexanediol, 50 mM Tris, pH 7.2

Temp: Ambient

Sanitization pH: >/= 12
Rinse conductivity: </= 0.5 mS/cm
Equilibrant pH: 7.0-7.4
Pooling: 40% to 35% UV

Collection/Pooling from: RPHPLC, IEXHPLC, HIHPLC

Resin Type: Agarose Weak Anion Exchange (DEAE-Sepharose FF)

Reverse-Phase pool loaded 1:1 with 6M Urea, 400 mM NaCl, 50 mM Tris, pH 7.2
Inline 0.2 µ filtration of load
Wash: 3M Urea, 200 mM NaCl, 50 mM Tris, pH 7.2; 2 CV

Sanitization pH: >/= 12
Rinse conductivity: </= 0.5 mS/cm
Equilibrant conductivity: 17.0-23.0 mS/cm
Equilibrant pH: 7.0-7.4
Collection/Pooling based on chromatogram (UV)

Membrane Type: Polyethersulfone (Millipore Biomax 5)
Molecular Wt. Cutoff: 5000 kD
Diafilter: 25 mM HEPES, pH 7.0
Diafilter volumes: 6

Sanitization pH: >/= 12
Rinse conductivity: </= 0.5 mS/cm
NWP: >/= 60% original
Manual integrity test: Pass
Equilibrant pH: 7.0-7.4
Target B-2036 Conc.: 7 mg/mL
Permeate pH: 6.9-7.1
Permeate conductivity: </= 1 mS/cm
Yield (w/DEAE): >/= 85%
DF Retentate: LAL, RPHPLC, Bioburden, DNA

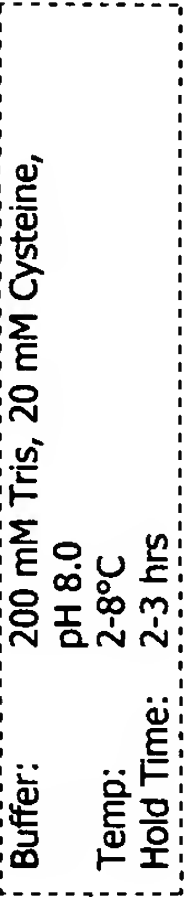
Figure 2 c

UNIT OPERATION

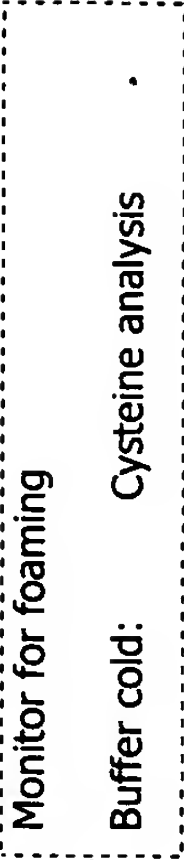
Retentate 1



PROCESS DESCRIPTION

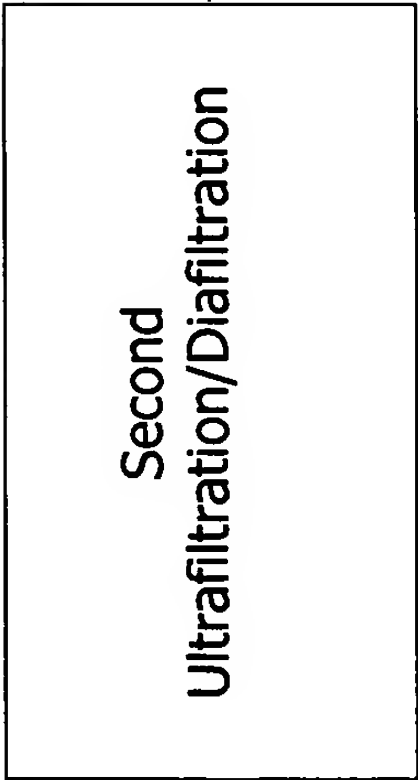


PROCESS CONTROLS

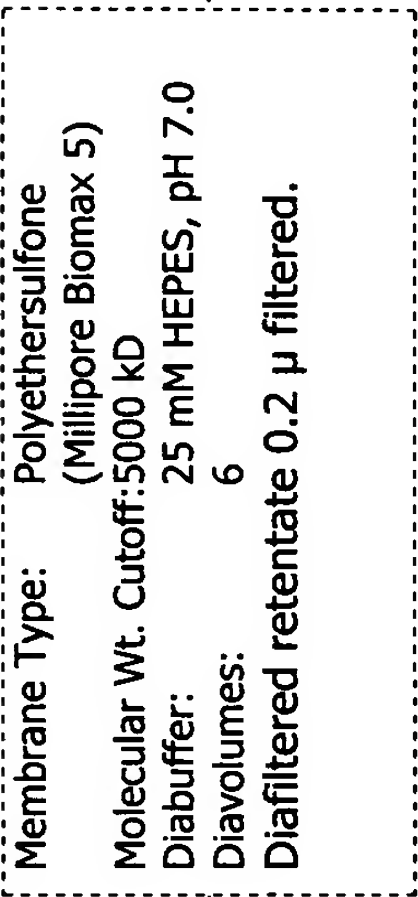


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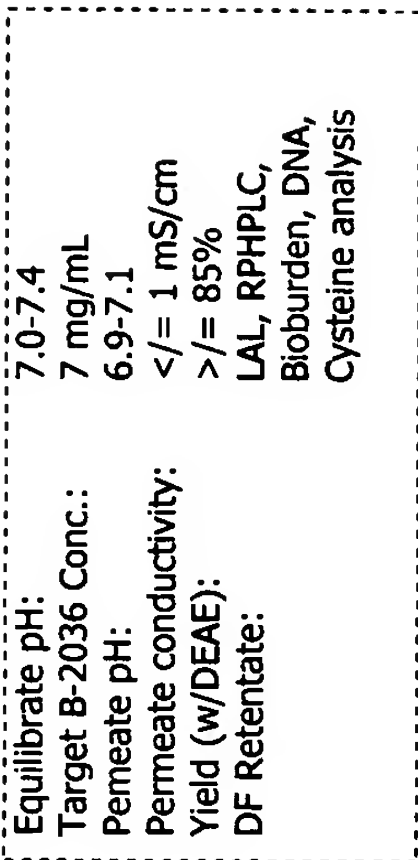
UNIT OPERATION



PROCESS DESCRIPTION



PROCESS CONTROLS

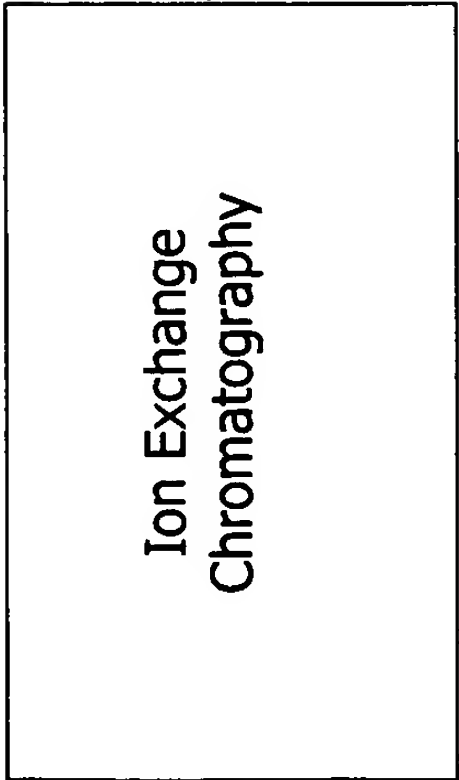


8.

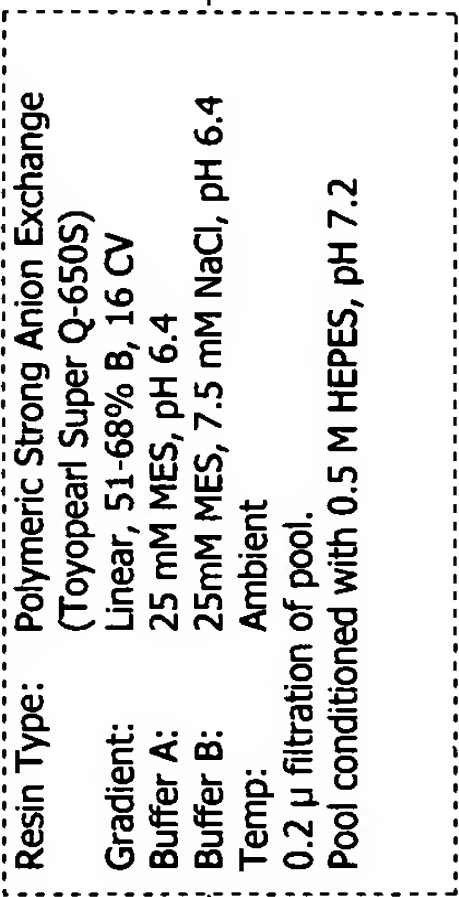
Permeate ? (Waste) 2



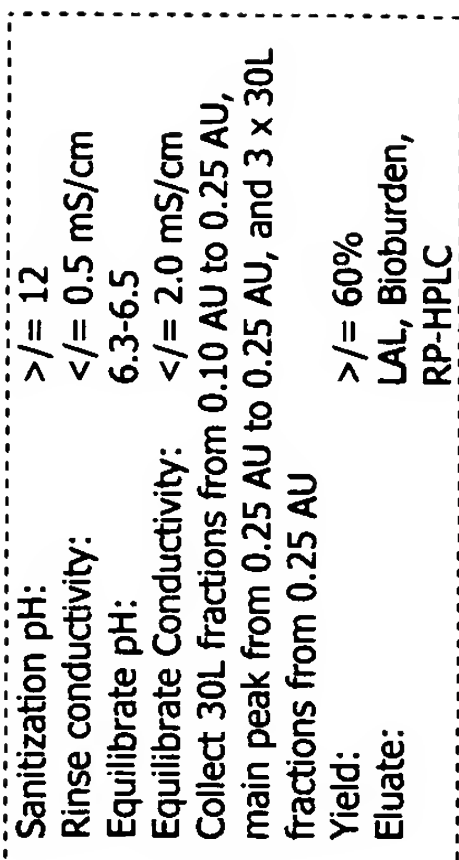
Retentate 2



PROCESS DESCRIPTION



PROCESS CONTROLS



9.

Figure 2 d

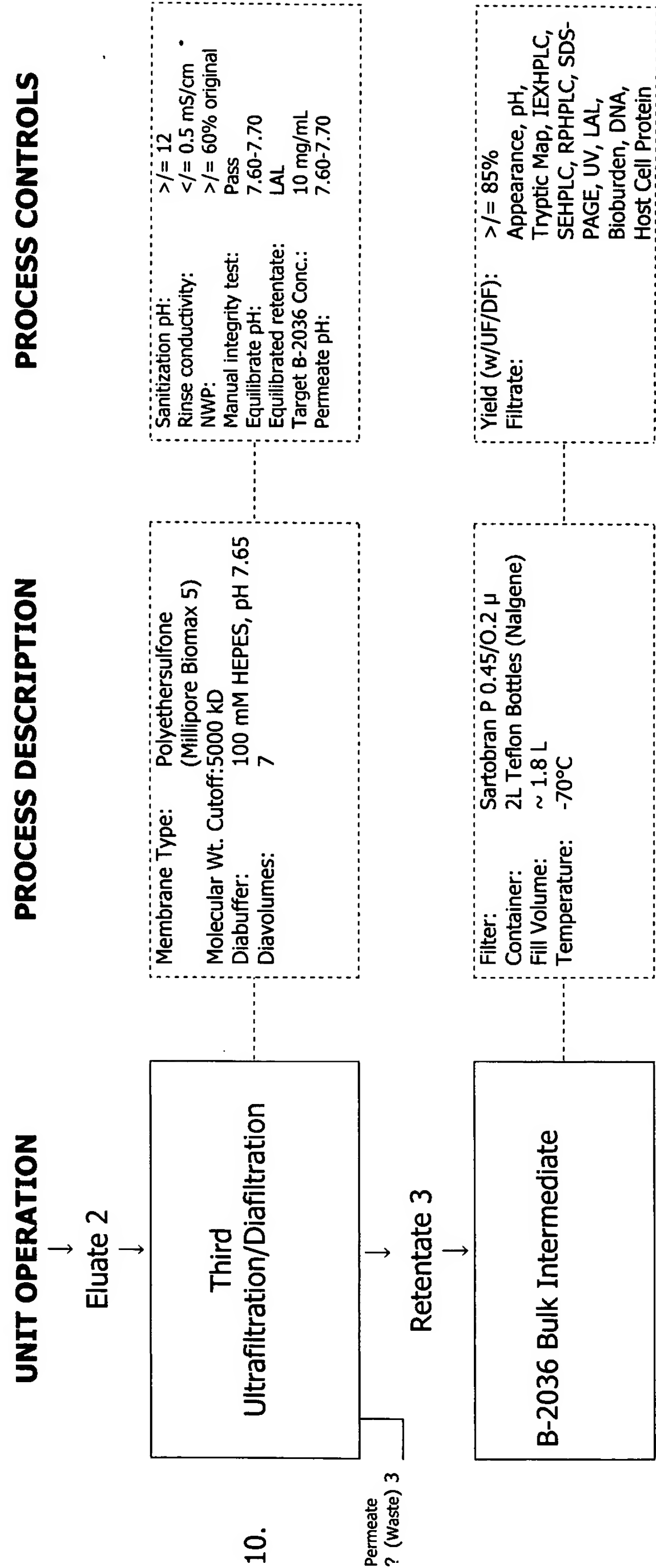


Figure 3 a

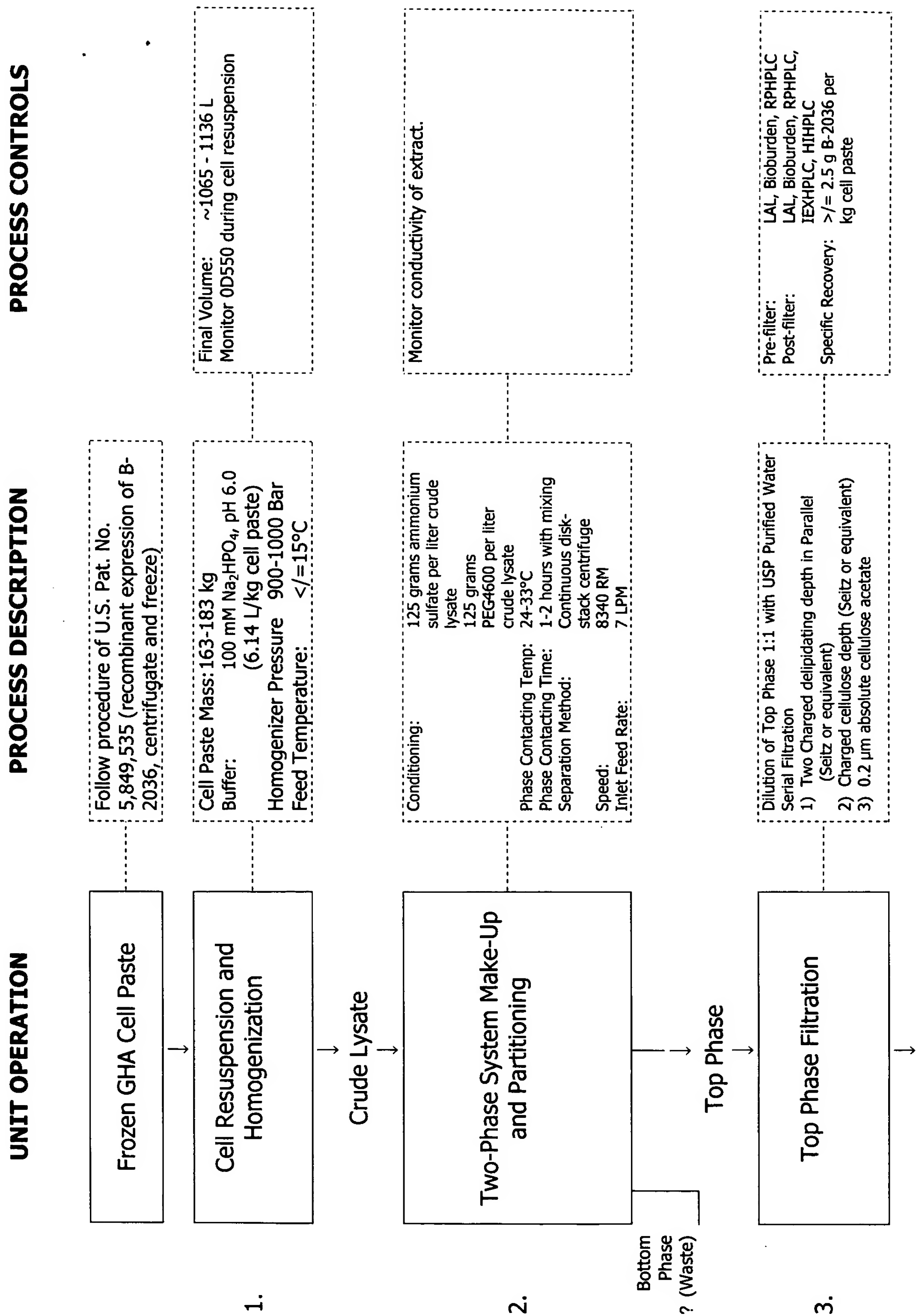


Figure 3 b

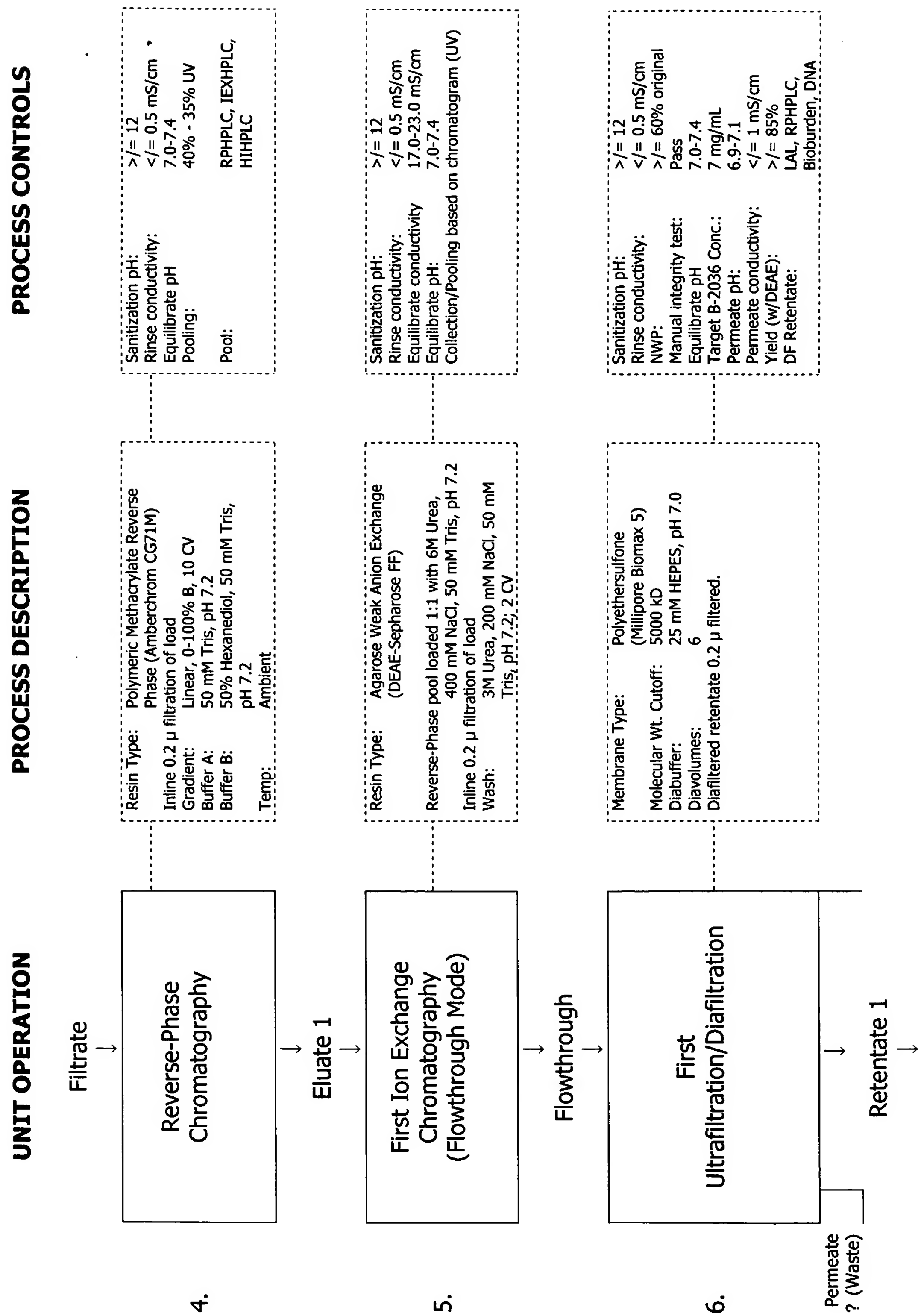


Figure 3 c

UNIT OPERATION

PROCESS DESCRIPTION

PROCESS CONTROLS

Ultrafiltration/Diafiltration
Retentate 1



Second Ion Exchange
Chromatography

Resin Type: Polymeric Strong Anion Exchange (Toyopearl Super Q-650S)
Gradient: Linear, 51-68% B, 16 CV
Buffer A: 25 mM MES, pH 6.4
Buffer B: 25mM MES, 7.5 mM NaCl, pH 6.4
Temp: Ambient
0.2 μ filtration of pool.
Pool conditioned with 0.5 M HEPES, pH 7.2

Sanitization pH: >/= 12
Rinse conductivity: </= 0.5 mS/cm
Equilibrate pH: 6.3-6.5
Equilibrate Conductivity: </= 2.0 mS/cm
Pooling: 0.25 to 0.25 AU
Eluate: LAL, Bioburden, RP-HPLC, IEX-HPLC, HI-HPLC, UV Scan



Eluate 2



Second
Ultrafiltration/Diafiltration

Membrane Type: Polyethersulfone (Millipore Biomax 5)
Molecular Wt. Cutoff: 5000 kD
Dibuffer: 100 mM HEPES, pH 7.65
Dialvolumes: 7

Sanitization pH: >/= 12
Rinse conductivity: </= 0.5 mS/cm
NWP: >/= 60% original
Manual integrity test: Pass
Equilibrate pH: 7.60-7.70
Equilibrated retentate: LAL
Target B-2036 Conc.: 10 mg/mL
Permeate pH: 7.60-7.70

Permeate
? (Waste)



Retentate 2



B-2036 Bulk Intermediate

Filter: Sartobran P 0.45/0.2 μ
Container: 2L Teflon Bottles (Nalgene)
Fill Volume: ~ 1.8 L
Temperature: -70°C

Yield (w/UF/DF): >/= 85%
Filtrate: Appearance, pH, Tryptic Map, IEXHPLC, SEHPLC, RPHPLC, SDS-PAGE, UV, LAL, Bioburden, DNA, Host Cell Protein